Product: Pembrolizumab
Protocol/Amendment No.: UVA Breast 48
V04-13-21

SPONSOR: Patrick Dillon

TITLE: Focused Ultrasound Therapy to Augment Antigen Presentation and Immune-Specificity of Checkpoint Inhibitor Therapy with Pembrolizumab in Metastatic Breast Cancer

IND NUMBER: 124939

SIGNATURE PAGE

Sponsor-Investigator		
_____	_____	_____
Name	**Signature**	**Date**

INVESTIGATOR'S AGREEMENT
I confirm that I have read this protocol and I agree to conduct the study as outlined herein. I agree to conduct the study in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices, as outlined in ICH E6, and the applicable laws and regulations.

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1.0 TRIAL SUMMARY

Abbreviated Title	Focused Ultrasound combined with Pembrolizumab in Metastatic Breast Cancer
Trial Phase	Pilot
Clinical Indication	Metastatic Breast Cancer
Trial Type	Investigator Initiated
Type of control	N/A
Route of administration	IV
Trial Blinding	N/A
Treatment Groups	Arm A: Pembrolizumab after High-Intensity Focused Ultrasound (HIFU) Arm B: Pembrolizumab before High-Intensity Focused Ultrasound (HIFU)
Number of trial subjects	Up to 15
Estimated enrollment period	2 years
Estimated duration of trial	3 years
Duration of Participation	90 days to 2 years
Estimated average length of treatment per patient	90 days

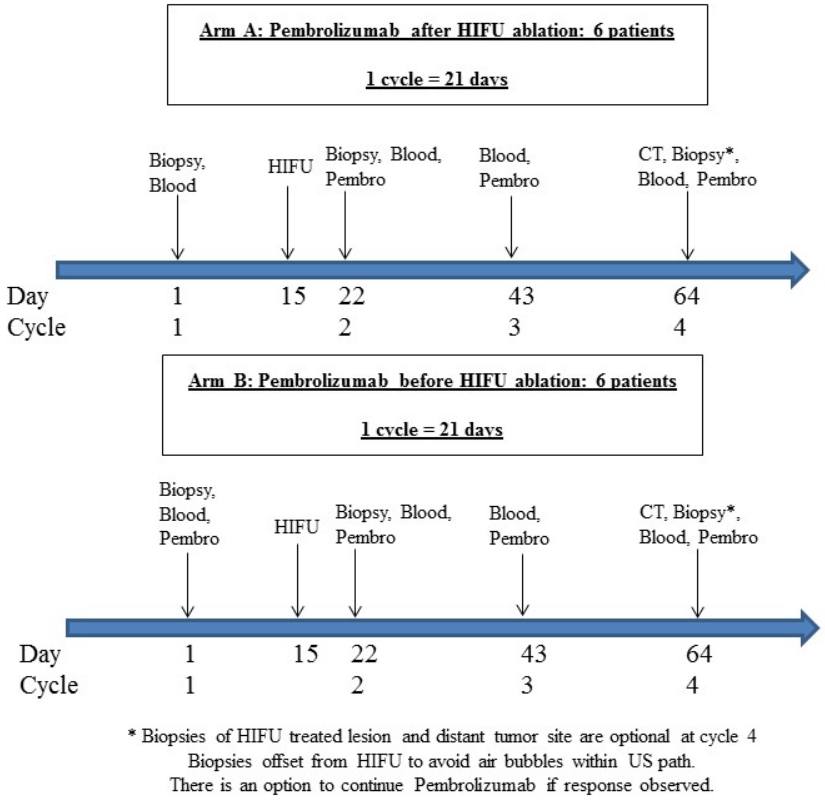
2.0 TRIAL DESIGN

2.1 Trial Design

This is a two-arm pilot study of High-Intensity Focused Ultrasound (HIFU) and immunotherapy. The goals of the study are to obtain preliminary data on the safety of HIFU with immunotherapy, delivered concurrently, and to obtain preliminary estimates of change in T-cell frequency and activation for HIFU alone (day 15-21 of ARM A) and pembrolizumab and HIFU given in combination (day 15-21 of arm B). The central hypothesis is that focused energy induced immunogenic cell death will overcome limitations in antigen availability, and effective T-cell responses, and thereby enhance immune-therapy of advanced breast cancer. Our secondary hypothesis is that concomitant immune-activation will further augment $CD8^+$ T-cell responses to tumor antigen released by HIFU. We will obtain preliminary data on whether HIFU promotes the presence of $CD8^+$ T-cells in ablated primary tumors, blood and non-ablated distant metastases. Additional correlative science endpoints will be tested as exploratory endpoints.

Eligible subjects will be randomized 1:1 to the investigational treatments (Arm A) or (Arm B). Subjects on both arms will undergo HIFU treatment on day 15. Subjects randomized to Arm A will receive pembrolizumab administered IV every 3 weeks on days 22, 43, and 64. Subjects randomized to Arm B will receive pembrolizumab administered IV every 3 weeks on days 1, 22, 43 and 64. In all subjects, restaging breast cancer-dedicated chest, abdomen and pelvic CT scan will be performed at baseline and cycle 4. Biopsies of tumors treated with HIFU will be completed on all patients at days 1 and 22 with optional distant tumor site biopsies and additional biopsies of the HIFU treated breast lesion to be completed at day 64. The tissue removed at the biopsies will be evaluated as part of the immunologic correlative studies.

2.2 Trial Diagram



* Biopsies of HIFU treated lesion and distant tumor site are optional at cycle 4
Biopsies offset from HIFU to avoid air bubbles within US path.
There is an option to continue Pembrolizumab if response observed.

3.0 OBJECTIVES & HYPOTHESES

3.1 Primary Objectives & Hypotheses

Objective: To determine whether the addition of pembrolizumab to HIFU increases the proportion of $CD8^+$ tumor infiltrating lymphocytes (ratio $CD8^+/CD4^+$) in the primary ablation zone of advanced or metastatic breast cancer.

Hypothesis: Focused energy induced immunogenic cell death is hypothesized to improve antigen availability, dendritic cell activity, effective T-cell responses. In combination with pembrolizumab, it is hypothesized that $CD8^+/CD4^+$ ratio in the ablation zone will increase by 25%.

3.2 Secondary Objectives & Hypotheses

Objective: To assess the adverse event profile of pembrolizumab and HIFU

Hypothesis: The combination of pembrolizumab and HIFU will result in adverse event profiles similar to previously reported profiles for each modality given alone.

3.3 Exploratory Objectives

(1) Objective: To compare $CD8^+$ T-cell responses at peri-ablation zones when pembrolizumab is given before or after HIFU. ($CD8^+/CD4^+$ ratios compared between arms)

(2) Objective: To evaluate clinical responses at local and distant metastatic sites by CT scan as measured by RECIST 1.1.

(3) Objective: To estimate the progression free survival (PFS) of breast cancer subjects treated with HIFU in combination with pembrolizumab.

(4) Objective: To estimate the overall survival (OS) of subjects with breast cancer treated with HIFU in combination with pembrolizumab.

(5) Objective: To evaluate immune responses to HIFU plus pembrolizumab by flow cytometric, immunohistochemical, cytokine, or other methods.

4.0 BACKGROUND & RATIONALE

4.1 Background

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on MK-3475.

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of $CD8^+$ T-cells and the ratio of $CD8^+$ effector T-cells / $FoxP3^+$ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral $CD4^+$ and $CD8^+$ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8-

(double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) is approved in the United Stated for the treatment of unresectable or metastatic melanoma, certain metastatic non-small cell lung cancers, and recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) with disease progression on or after platinum-containing chemotherapy.

4.1.2 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

4.2.1 Breast Cancer

Breast Cancer remains the most common non-skin cancer in women and remains the number two cause of cancer mortality in women in the United States. Metastatic breast cancer is incurable with a 5 year overall survival of 22%. There are no curative therapies for metastatic breast cancer at this time. Nevertheless, immunotherapies in breast cancer and other solid tumors have provided long-term disease-free intervals of clinical benefit. Indeed, some breast cancers generate immune recognition, though complete immunologic rejection remains rare at least for breast cancers (1, 2). It is thus not surprising that spontaneous regressions are rare for breast cancer. Accordingly, the density of NK and cytotoxic T cell infiltrates within breast tumors is generally low, compared to melanoma or renal cell carcinoma (3, 4). Furthermore the circulating NK and T cells display significant dysfunction in the setting of breast cancer (5). Due to these concerns, breast cancer has not received much attention for immunotherapy until recently. We now know that dampened immune responses in breast cancer are partly due to release of type 1 interferons, the recruitment of myeloid derived suppressor cells (MDSC's), recruitment of T regulatory cells and expression of PD-L1 on some breast carcinomas (1, 2). Recent clinical data indicates that checkpoint blockade and other immunotherapies can break the immune privilege enjoyed by many breast cancers (6, 7). Further, Cimino-Mathews et al. elegantly demonstrated that treatment-naive PBCs contain both PD-L1$^+$ TILs (78% of studied tumors) and PD-L1$^+$ carcinoma cells (21% of tumors) which were primarily localized to the invasive fronts of tumor cell nests (2). Further PD-L1$^+$ carcinomas were more likely to contain PD-L1$^+$, CD3$^+$, CD4$^+$, CD8$^+$, and FoxP3$^+$ T cells, but not B cells. They also observed that all PD-L1$^+$ primary breast cancers were

associated with peripheral lymphoid aggregates, where less than half of PD-L1 negative primary breast cancers had lymphoid aggregates.

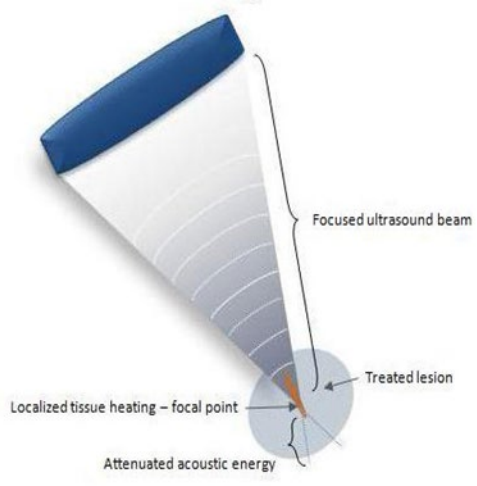
Clinical experience with immune modulators in breast cancer has been encouraging in the triple negative subset of patients and patients with pre-existing immune infiltrates (1, 6-8). There is modest clinical outcome and survival data for immunotherapy in breast cancer as noted in the Nanda trial and from abstracts at national meetings (7, 9, 10), but data from large, randomized studies is not yet mature.

Immunotherapy, including PD-1 blockade, is thought to prime the immune system more effectively when tumor antigens are released in the context of a locally stimulating microenvironment rather than in tolerogenic environments. For example, a CTLA4 inhibitor performed better against solid tumors when given with local ablation by radiation (11) and with cryotherapy (12, 13). Additionally, there are several reports of superior performance of combinations of immune modulators over single modality therapies (i.e., vaccines, TLR agonists, cytokines, checkpoint inhibitors, etc.) Several combination trials of immunotherapy with radiation, chemotherapy, hormonal therapy or tumor ablation are ongoing.

4.2.2 Rationale for the Trial and Selected Subject Population

High Intensity Focused ultrasound (HIFU) is an ablative therapy which can heat tumors (2mm-5cm) rapidly to cell-lethal temperatures and simultaneously perturb the microenvironment, the microvasculature, and the lymphatics. At typical energy levels, HIFU can preferentially induce controlled apoptotic cell death rather than liquefactive necrosis. HIFU does not involve radiation and is not believed to be mutagenic. Instead, heat shock proteins, cytokine release and cellular mediated mechanisms predominate and are documented to result in systemic $CD8^+$ T cell recognition of tumor antigens (14). HIFU has been demonstrated to be an effective method for inducing antigen exposure and presentation to dendritic cells, thus rending HIFU an auto-vaccine in some regards (15, 16).

HIFU ablation of a single site of disease administered in combination with systemic immunotherapy may achieve control of systemic disease. Such a response would be tantamount to the described abscopal effect. In autochothonous mouse models, our group showed additive effects of combining HIFU with various immunotherapies (unpublished). We have also studied HIFU for drug delivery in several ongoing HIFU studies using devices with IDE clearance. Other immune stimulating effects of HIFU have been recently reviewed by Unga and Wu (14, 17).



The University of Virginia is an internationally recognized center for focused ultrasound research in pre-clinical and clinical studies. We are currently conducting the first clinical trial of the Theraclion HIFU device in the US for the treatment of breast fibroadenoma. No serious AE's have been seen in over 200 patients treated with the Theraclion HIFU device in Europe.

Positive results from the MSKCC clinical study of cryotherapy plus ipilimumab in early stage breast cancer (presented at ASCO 2014) further support the development of combinatorial studies for the treatment of breast cancer. These data and experience in a phase Ib metastatic breast cancer study

provide a strong rationale for the immune stimulating role of pembrolizumab in breast cancer (7). Furthermore, there is encouraging safety data for combinations of pembrolizumab with various chemotherapeutic agents, radiation and surgery. We propose that the combination of HIFU and pembrolizumab will be safe and synergistic to allow better antigen presentation, immune recognition of breast cancer and possibly even result in improved disease control at local and distant sites of disease.

The inclusion of patients with advanced metastatic disease is justified because there are no curative therapies for metastatic breast cancer and the existing therapies have intolerable toxicities. Additionally, the inclusion of patients who experience disease progression by rising tumor marker is in alignment with current standards of care in metastatic breast cancer. Indeed, the earlier that progressive disease is identified, the more likely that patients will benefit from a later line of therapy and the less likely that a given patient will experience a major complication due to bulky disease. The same rationale is applied to minor subset of patients who become intolerant of endocrine therapies. When intolerance occurs, the existing standard of care is to switch to cytotoxic therapies, but occasional patients will elect treatment holiday rather than risking treatment with a cytotoxic agent. Thus, the sub-population of endocrine intolerant metastatic breast cancer patients should also be afforded the same opportunities to participate in clinical trials (see inclusion criteria number 4).

4.2.3 High Intensity Focused Ultrasound (HIFU)

The use of high intensity focused ultrasound (MRI guided or ultrasound guided) has been tested in different types of soft tissue cancer such as prostate cancer, liver cancer and breast cancer (18). High Intensity Focused Ultrasound (HIFU) is a non-invasive alternative to surgery. In HIFU, high-energy ultrasound is used to deliver a large amount of sound energy to a focal point to rapidly induce tissue heating to 85° - 90°C. This initiates tissue coagulation followed by tissue necrosis to ablate the targeted area. To minimize non-target tissue damage, the ultrasound is focused using MRI or ultrasound. This study proposes to use an ultrasound-focused HIFU device, the EchoPulse (Theraclion). Echopulse is a computer-driven system comprised of an Electronics Cabinet, an extra-corporal probe (VTU – Visualization Treatment Unit) mounted on an arm and moved by motors, a cooling unit (EPack) and an ultrasound imaging scanner. This device has been used in prior European studies of head and neck pathologies, including thyroid and parathyroid nodules, and in breast fibroadenoma (32, 33, 34, 35). Echopulse is CE marked by Theraclion for treating nodules within the thyroid and parathyroid glands and for breast fibroadenomas.

4.2.4 HIFU in Head and Neck Malignancies

Since November 1st 2007, EchoPulse (Theraclion) has been CE marked for HIFU treatment of neck pathologies. 11 patients with primary hyperparathyroidism have been treated and followed in a clinical trial. The mean follow-up is 9.5 months (1-28 months).

The Echopulse device has been tested in patients (n=25) scheduled for thyroidectomy for multi-nodal goiter (19). Prior to thyroidectomy, HIFU was applied to a single thyroid nodule and the effect on size and function were evaluated pre- and post-treatment. Three patients were discontinued due to adverse events and 22 patients completed the study. Reductions in node volumes were observed in 16 of 22 patients (2 – 80% reduction). This pilot study was extended to include patients with benign thyroid nodules. Overall, 21 patients were treated with HIFU versus 11 untreated patients in the control group. The follow up of this study has been completed. No serious adverse events (SAE) were reported. The analysis is currently available (19).

The following side effects have been observed in patients with the HIFU device for primary hyperparathyroidism:

- Mild to moderate pain during treatment
- Potential fatigue following the treatment
- Edema of the subcutaneous tissue which recovered within up to one month without any directed treatment.
- Dysphonia which may last up to 3 months. Normal voices were recovered in all cases.
- Mild to moderate decrease in the mobility of the vocal cord on the same side of the gland treated. This lasted for up to 6 months in rare cases. All cases were transient.

4.2.5 HIFU in Breast Fibroadenoma

EchoPulse was first evaluated for the treatment of breast fibroadenomas between May 2011 and February 2013 in a pilot feasibility study performed in four sites in France and Bulgaria. Fifty-one fibroadenomas in 42 patients were treated. The average treatment time was 1h12min (0h26-2h33). Volume reductions are described in **Table 1:** Volume reduction over time. The HIFU treatment was well tolerated. In 36% of the cases, post-treatment skin irritation and erythema were observed, although both toxicities spontaneously resolved 1 month following treatment (20).

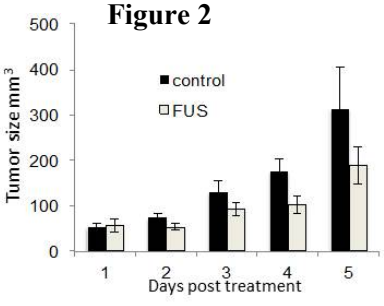
Table 1: Volume reduction over time

Follow-up period (months)	Patients (n)	Mean volume reduction (%)
M2	39	33.6±19.2
M4	33	48.7±22.7
M6	26	57.5±15.7
M9	11	66.2±11.9
M12	7	68.8±11.4
M18	2	69.2±5.0

4.2.6 Rationale for Combination

The goal of the proposed study is to establish the feasibility, safety, and effectiveness of non-invasive HIFU therapy in combination with pembrolizumab for the treatment of metastatic breast tumors. To date, there have been publications of excellent response rates to PD-1 antibody therapy in triple negative breast cancer, but the responses in estrogen receptor positive breast cancer have been lower.

Figure 2



In order to extend immunotherapy to a broader spectrum of the breast cancer population (such as estrogen receptor positive tumors), we propose to combine pembrolizumab with a local tissue injury to enhance antigen presentation to dendritic cells and render the microenvironment more stimulatory to immune activation (21). It is known that an insult to a part of a tumor leads to an increase in activated, specific T cell responses to newly exposed tumor antigens throughout the whole tumor (22). Sub-total thermal injury thus acts as an autologous vaccine, especially in the sub-lethal zone around an ablation site (23). Indeed, CTLA4 inhibition with cryoablation had an additive immune effect in breast cancer (24).

We propose the use of a non-invasive thermal ablation combined with pembrolizumab to assess immune synergy. Our ablation modality of HIFU is a safe and readily available technology for delivery of both thermal and mechanical tissue disruption for multiple tumor types. HIFU can heat tumor rapidly to 45-90°C. We tune HIFU power and patterns to induce controlled apoptotic cell death rather than liquefactive necrosis (25). Thus HIFU treatment of tumors results in heat shock protein responses, cytokine release, dendritic cell (DC) maturation, migration as well as T and NK cell activation and trafficking (25-29). We show here in Figure 3, our group's work on HIFU in mouse studies.

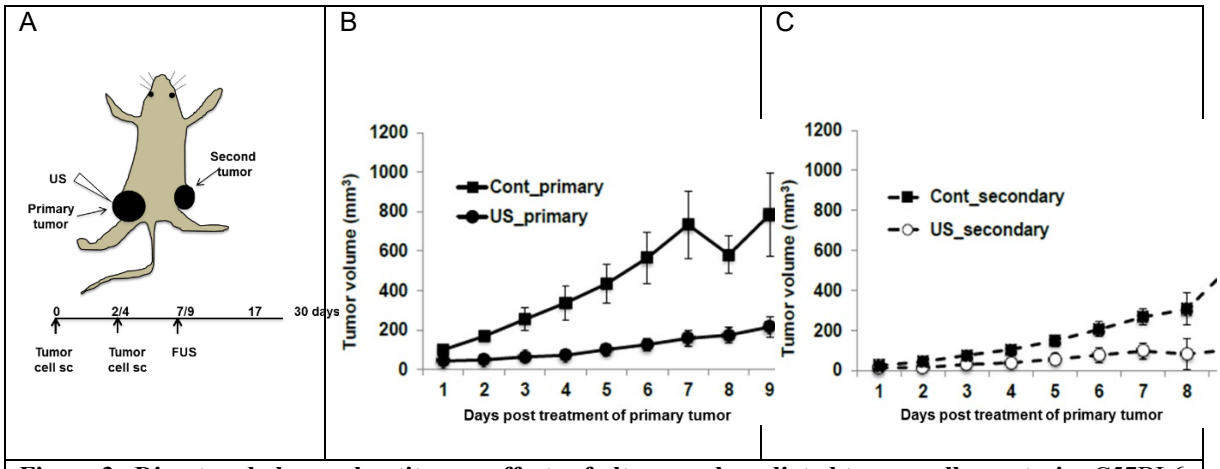


Figure 3. Direct and abscopal antitumor effects of ultrasound-mediated tumor cell apoptosis. C57BL6 mice bearing 2 subcutaneous B16 melanoma deposits, on the left flank (primary tumor) and right flank (secondary tumor), were treated with ultrasound (1.0 MHz, ~1.0 MPa, 100 burst count every 5 seconds, 10 min duration) at the "primary" tumor **(A)**. Control mice were not treated. The growth of each tumor was measured over time after treatment (or control) of the primary tumor. US treatment of the primary tumor led to marked slowing of growth of the primary tumor **(B)**, and also led to marked growth delay of the "secondary" tumor in the same mouse **(C)**. (unpublished data from Kumari Andarawewa)

It is observed that sub-ablative tumor disruption by FUS is novel and immunologically relevant. Sub-ablative FUS treatment results in recognition of a larger array of epitopes than surgery in clinical and preclinical studies (16, 25, 27). FUS combined with immunotherapy in mice results in increased myeloid, NK and T cell responses locally and systemically compared to either modality alone (20, 25-30). FUS alone was shown to induce immune response in tumors and specifically, sub-ablative FUS achieved controlled apoptosis with efficient antigen delivery to DC's; NK and TIL infiltrations were also enhanced (25, 26, 29, 31). FUS is also believed to help prevents T cell tolerance (15). FUS stimulates T cell infiltration into breast tumors as shown by studies examining the microenvironment (15, 16, 32, 24, 25).

Additionally, HIFU may enhance drug delivery to the tumor site. FUS increases capillary leakage and local chemokine release, thus augmenting drug delivery and T-cell trafficking (25, 33-35). Additional rationale is that anti-PD-1 antibodies and FUS have largely non-overlapping toxicities. The toxicities for both treatments are minimal. FUS is non-invasive and performed with mild sedation (20, 30, 31).

We are currently studying HIFU in breast fibroadenomas and bone metastases from any solid tumor (NCT02078011 and NCT00656305). No serious AE's have been seen in over 200 patients treated with HIFU for breast diseases to date. HIFU is safer and easier to contour than other ablative therapies. Other therapies produce spherical ablation zones, whereas HIFU can be contoured and carefully tuned to sub-ablative power for individual tumors. Patients and providers are motivated by these technical improvements (27).

In this pilot study, we will test pembrolizumab therapy in combination with HIFU to assess for enhanced immune stimulation and antitumor effects at local ablation sites, distant, non-treated sites and in the blood. Biopsy data from before and after therapy with pembrolizumab and HIFU will demonstrate both immunologic and tumor responses to the combined therapy proposed. We will examine the tissue in the peripheral zone of ablation as well as distant metastatic sites for CD8 and CD4 T cells with additional analyses of MDSC's, T-regulatory cells and cytokine responses as as tissue quantity permits.

4.3 Rationale for Dose Selection for Pembrolizumab

The planned dose of pembrolizumab for this study is 200 mg every three weeks (Q3W). Based on the totality of data generated in the Keytruda development program, 200mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies in melanoma and NSCLC indications demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg Q2W, representing an approximate 5- to 7.5-fold exposure range (refer to IB, Section 5.2.2)
- Population PK analysis showing that both fixed dosing and weight-based dosing provides similar control of PK variability with considerable overlap in the distributions of exposures, supporting suitability of 200 mg Q3W
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

4.4 Rationale for Endpoints

This study is designed primarily to test whether interfering with immune suppression by blocking the molecular interaction between the PD-1 receptor and its cognate ligand, PD-L1, increases the number and activation of TILs that are induced by the local ablative therapy.

The study is also designed to examine the safety of HIFU and pembrolizumab. HIFU therapy for breast lesions and breast cancer is generally well tolerated. Based on our experience at UVA (n=20) there has been no evidence of grade 4 toxicities in patients who received HIFU. Pembrolizumab has been studied in numerous clinical trials to date and was found to be well tolerated with no dose-limiting toxicities at the standard dose of 200 mg (see Investigator's Brochure). Our proposed study is the first study to combine HIFU and pembrolizumab in humans and safety is our secondary endpoint.

4.4.1 Biomarker Research

This study will examine the effect of pembrolizumab in combination with HIFU on select immune mediators by estimating the difference in post-treatment expression of tissue immune biomarkers for two treatment groups. In addition, blood samples collected at various time points pre- and post-

treatment will allow us to evaluate the effect of this treatment on different subsets of circulating immune cells. Tissue quantity may limit some assays and therefore biomarker examinations are prioritized as indicated in Section 7.1.2.8.1.

CD4/CD8 infiltrating effector T cells (TILs)
As described in the background, the CD4/CD8 infiltrating effector T cells (TILs) are not frequently observed to be present in breast cancer, however, when present they correlate with better outcomes (36). HIFU is one modality that is suggested to increase homing of T cells to cancer sites (37). Our hypothesis is that targeting the host immunosuppression by blocking the PD-1/PDL-1 interaction may enhance the number or activity of TILs and potentially improve the outcomes of metastatic or unresectable breast cancer patients.

T-Cell activation markers
When activated, T-cells start to express activation markers such as CD45RO, CD57, CD69, Granzyme B, Ki67, and MHC-1. In order to study the effect of pembrolizumab plus HIFU on the activation of effector T-cells, expression of these activation markers in the biopsy samples and peripheral blood will be assessed at baseline and at the day 22 biopsy. Similarly, peripheral blood will also be analyzed at all the time points listed in trial diagram in section 2.2. Additional markers may be added or subtracted during the course of analysis as deemed necessary.

Myeloid markers
A major mechanism of resistance for immunotherapy in breast cancer is thought to be tumor associated macrophages and myeloid derived suppressor cells. A standard myeloid panel containing CD11b, CD33, HLA-DR, CD14 and CD15 is planned for flow cytometric analysis of tumor lysates. Additional IHC staining using these markers may be performed if adequate tissue is available.

T regulatory cells (T-regs)
T regulatory cells ($FoxP3^{+}CD4^{+}CD25^{+}$) are another subset of tumor infiltrating lymphocytes (TILs) but they are known for their immunosuppressive activity. Patients with breast tumors that have low T-regs were found to have better survival compared to tumors with high T-regs (38). Accordingly, the effect of adding pembrolizumab to FUS on the number of T-regs may be assessed in the tumor tissue and the peripheral blood depending on tissue availability.

PD-L1 expression on breast tumor
The expression of PD-L1 in breast cancer was reported in 8% to 38% of surgical samples and was found to negatively affect survival (39-41). Here we hypothesize that adding pembrolizumab will block the interaction of PDL1/2 with PD-1, releasing the break on the immune system and leading to an enhanced immune response against the breast cancer. Accordingly, the expression of PD-L1 in breast cancer tissues may be assessed in this trial if adequate tissue remains after primary biomarker analyses are exhausted.

PD-1 expression on TILs and PBMC's
The program cell death (PD-1) is induced on T cells, B cells, and monocytes on activation and it interacts with its ligand PD-L1 to down modulate the immune system. It is of interest to evaluate how blocking the PD-1/PD-L1 interaction may affect the expression of PD-1 on T-cells. Since PD-1 assays may be complicated by the presence of pembrolizumab, PD-1 may be assessed by a gene expression assay (qRT-PCR) on PBMC's and/or tumor biopsy samples.

Chemokines
This study may include additional analysis of chemokines and homing ligands on endothelial cells as a function of HIFU. Trafficking of activated T cells to treated tumors is anticipated to be augmented by both HIFU and the application of pembrolizumab. Potential assays for breast biopsy tissue may include, but are not limited to the following: CXCR4, CXCL12, CCL18 (co-assay with CD68), CXCL9, CXCL10, IL-10, and TGF-β. Potential assays for any bone biopsies may include: CCL2, CX3CL1, CX3CR1, IL-10 and TGF-β.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

- Subjects with histologically confirmed metastatic or unresectable breast cancer (male or female)

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent/assent for the trial.

2. Must be determined to have metastatic or unresectable disease, as determined by treating physician. (Must have at least evaluable disease, but does not need to be measurable disease by RECIST 1.1)

3. Any receptor status (estrogen receptor, progesterone receptor, HER2 receptor). Patients who are HR^+ should also no longer be candidates for hormonal-based therapy. Patients who are $HER2^+$ should have progressed on or no longer be candidates for available HER2 directed therapy. Hormonal therapy must be stopped prior to day 1 of treatment.

4. Patients must have had at least one prior line of therapy for breast cancer in the metastatic/unresectable setting. Patients demonstrating a rising tumor marker during a line of therapy or demonstrating intolerance of a standard or investigational therapy meet this criteria (includes endocrine or chemotherapy or targeted therapy).

5. Patients must have an accessible lesion in the breast/chest wall/axilla which has not been previously thermally ablated. Prior breast irradiation is acceptable if the lesion has recurred or grown following radiation.

   The chest wall is defined per the National Cancer Institute at the National Institutes of Health (NCI) as: the skin, fat, muscles, bones, and other tissues that form a protective structure around vital organs in the area between the neck and the abdomen, including the heart, major blood vessels, lungs, and liver. The bones in the chest wall include the ribs, sternum (breastbone), and spine. The chest wall also helps support breathing and movement of the upper arms and shoulders.

"https://www.cancer.gov/publications/dictionaries/cancer-terms/def/chest-wall" [Accessed 5/7/2019]

6. Subjects must have at least one target lesion in breast/chest wall/axilla which is amenable to application of high intensity focused ultrasound:
    a. The distance from the skin: A targetable portion of the tumor must be ≥ 5 mm from the skin
    b. the rib cage should not be in the prefocal ultrasound path or behind the target area of the lesion (minimum distance from the posterior aspect of the target area to rib cage must be at least 10 mm).
    c. subject's tumor must be larger than 9 mm in the anterior-posterior dimension (measured by ultrasound).
    d. subject's tumor must be greater than or equal to 0.3 cubic centimeters.
7. Subject must be ≥ 18 years of age on day of signing informed consent.
8. Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion.
9. Have a performance status of 0-2 on the ECOG Performance Scale.
10. Demonstrate adequate organ function as defined in Table 2.

Table 2: Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

[a]Creatinine clearance should be calculated per institutional standard.

11. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication or study indicated ultrasound treatment. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

12. Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

13. Male subjects of childbearing potential (Section 5.7.2) must agree to use an adequate method of contraception as outlined in Section 5.7.1- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Systemic corticosteroids of less than 10 mg per day of prednisone (or equivalent) are allowed. Topical corticosteroids are acceptable, including steroids with very low solubility administered nasally for local effects only (e.g. Nasonex®).

3. Has a known history of active TB (Bacillus Tuberculosis)

4. Hypersensitivity to pembrolizumab or any of its excipients.

5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

6. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

- Note: If subject received major surgery or radiation, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

- Note: anti-estrogen therapy must be stopped prior to study Day 1.

7. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging within four weeks prior to registration and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

9. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

10. Has a history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or current pneumonitis/interstitial lung disease.

11. Has an active infection requiring systemic therapy.

12. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

13. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

14. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

15. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent within the prior 24 weeks.

16. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

17. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

18. Has received a live vaccine or live attenuated vaccine within 30 days of planned start of study therapy. Administration of killed vaccines is allowed.

Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

19. HIFU must not be applied to a breast with an implant. A region outside of the breast may be targeted as long as the targeted area is at least 10mm away from an implant.

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 3: Trial Treatment

Table 3: Trial Treatment

Arm	Drug	Dose	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
A	Pembrolizumab	200 mg	Q3W	IV infusion	Days 22, 43, 64 and Day 1 of each ensuing 3 week cycle	Experimental
B	Pembrolizumab	200 mg	Q3W	IV infusion	Days 1, 22, 43, 64 and Day 1 of each ensuing 3 week cycle	Experimental
A/B	Focused Ultrasound Tumor ablation[1]				Day 15	Experimental

[1]Focused Ultrasound ablation should target 50% of the tumor, up to 3 cubic centimeters, not the entire tumor volume. See eligibility criteria for minimum tumor size parameters. There is no maximum tumor size parameter. See also section 5.2.1.2.

Trial treatment should begin on the day of randomization or as close as possible to the date on which treatment is allocated/assigned.

5.2.1 Dose Selection/Modification & Focused Ultrasound Administration

5.2.1.1 Pembrolizumab Dose Selection:

The rationale for selection of doses to be used in this trial is provided in **Section 4.0** – Background and Rationale.

The commercial Pembrolizumab product provided by Merck will be prepared and administered per standard practice.

5.2.1.2 Focused Ultrasound Administration

HIFU will be administered to sites of disease located in the breast/chest wall/axilla. The treatment will be delivered using local anesthesia, conscious sedation with local anesthesia, or general anesthesia. The choice of anesthesia will be determined by the treating physician for each individual patient. The patient will be placed in the lateral decubitus position, depending on the side of the tumor with the arm placed over the patient's head. The breast is then immobilized. To mitigate damage to the rib cage, the clinician will confirm that the rib cage is not in the prefocal ultrasound path or behind the focal point (minimum distance behind the focal point = 10 mm). As the risk of HIFU on the lung parenchyma is not known at this time, for those patients treated with chest wall lesions the beam energy should always be oriented so that it does not reach the lung. Based on the images recorded at the inclusion visit, the physician

performing the therapy will perform the ultrasound (US) localization of the targeted area of the tumor (approximately 50% of the tumor volume, up to 3 cubic centimeters total ablated volume).

The treatment head that will be used has a focal length of 38 mm.

The treatment procedure of Echopulse consists of six treatment steps:
- Pre-treatment ultrasonography
- Positioning
- Planning
- Energy setting
- Generation of HIFU treatment pulses in the volume defined above
- Post-treatment visualization and final report.

The targeted area for ablation and final assessed ablation parameters of the HIFU treatment will be recorded in the CRF.

When a HIFU session is prematurely interrupted, the reason of interruption will be recorded in the CRF (adverse event, technical failure, others). Due to the wide variability in tumor size, shape, depth, subcutaneous fat distributions, and vascularity, the energy settings will necessarily vary between patients, but effort will be made to keep ablation target zone close to the 50% target range and a maximum of 3 cubic centimeter area.

Following the HIFU procedure, local application of ice may be applied to the affected area.

5.2.2 Dose Modification

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 4 below. See Section 5.6 for supportive care guidelines, including use of corticosteroids.

Table 4: Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab Monotherapy and IO Combinations

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Study intervention must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last study intervention treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If study intervention has been withheld, study intervention may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up

Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea/Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Recurrent Grade 3 or Grade 4	Permanently discontinue		
AST or ALT Elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg	

			prednisone or equivalent) followed by taper	
T1DM or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer antihyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		

Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 to5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs (eg, vasculitis and sclerosing cholangitis).

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record

Note: There is no dose modification for HIFU as it is only administered once in this protocol.

5.2.3 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Staff should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

The commercial Pembrolizumab product provided by Merck will be prepared and administered per standard practice.

5.2.4 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

5.3 Randomization or Treatment Allocation

Randomization will be discussed with participants during the process of informed consent and will occur after registration and within 7 days prior to the start of treatment. The randomization codes are generated by the study statisticians and stored in the Cancer Center Clinical Trials Database.

5.4 Stratification

No stratification planned.

5.5 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

5.5.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

5.5.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management (except glucocorticoids for irAE's) should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.6 Rescue Medications & Supportive Care

5.6.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**:

  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Hypophysitis**:
  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:

  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:

- For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
  - Treat with IV or oral corticosteroids
- For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
- When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

  Table 5 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 5: Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
(e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

5.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence[†] from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.2.5.

5.7.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.5 – Other Procedures.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression

  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 5.8.1
- Unacceptable adverse experiences as described in Section 5.2.2.
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 7.1.6.5.
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.6 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-

study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.8.1 Treatment after Initial Radiologic Progression

If radiologic imaging shows progressive disease (PD), tumor assessment may be repeated ≥4 weeks later in order to confirm PD with the option of continuing treatment while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued as per treatment calendar. If repeat imaging confirms progressive disease, subjects will be discontinued from study therapy. In determining whether or not the tumor burden has increased or decreased, investigators should consider all target lesions as well as non-target lesions, with the exception of radiated lesions, which will only be considered when evaluating increased tumor burden. The decision to continue study treatment after the first evidence of disease progression is at the Investigator's discretion and will be based on the clinical status of the subject. Subjects may receive study treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

Subjects that are deemed clinically unstable are not required to have repeat imaging for confirmation.

5.8.2 Discontinuation of Study Therapy after CR after Cycle 4

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 10 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.1.6.5.

5.8.3 Treatment with pembrolizumab after SD or PR

Treatment with pembrolizumab for subjects who have attained a SD or PR should continue every 3 weeks until the subject reaches a confirmed disease progression, achieves confirmed CR as per Section 5.8.2 or experiences an adverse event meeting criteria for discontinuation as per Section 5.8. For patients with long term stable disease or long term PR not meeting CR criteria, a subject may continue treatment for up to 2 years. Subjects experiencing SD or PR may elect to discontinue pembrolizumab treatment any time. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and

the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.1.6.5.

5.9 Subject Replacement Strategy

5.9.1 Replacement of Study Participants

A participant who is enrolled but who does not receive study drug or any of the study related procedures may be replaced. Every attempt will be made to evaluate any data from these participants for endpoint assessment.

5.10 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

- Quality or quantity of data recording is inaccurate or incomplete
- Poor adherence to protocol and regulatory requirements
- Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
- Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

ARM A

Trial Period:		**Treatment Cycles[a]**									**End of Treatment**	**Post-Treatment**		
		Cycle 1					May be repeated beyond 8 cycles							
Treatment Cycle:	Main Study Screening (Visit 1)	C1D 1	C1D 15	2	3	4	5	6	7	8		Safety Follow-up-30 days post discon	Follow-Up Every 12 weeks up to 2yrs[i]	Survival Follow-Up[n] Annually
Days:		1	15	22	43	64	85	106	127	148	Discon[h]			
Scheduling Window (Days):	see footnotes	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	± 3	±3wks	
Informed Consent	X													
Inclusion/Exclusion Criteria	X													
Demographics and Medical History	X													
Prior and Concomitant Medication Review	X	X		X	X	X	X	X	X	X	X	X		
Ultrasound	X													
Focused Ultrasound Treatment			X											
Pembrolizumab Infusion				X	X	X	X	X	X	X				
Post-study anticancer therapy status												X	X	X
Survival Status														X
Review Adverse Events		X	X	X	X	X	X	X	X	X	X	X[j]	X[j]	
Full Physical Examination	X[b]													
Pain Assessment (VAS Scale)			X	X										
Directed Physical Examination		X		X	X	X	X	X	X	X	X	X		
Vital Signs and Weight	X[c]	X		X	X	X	X	X	X	X	X	X		
ECOG Performance Status	X[b]	X		X	X	X	X	X	X	X	X			
Pregnancy Test – Urine or Serum β-HCG	X		X	X[f]										
PT/INR and aPTT	X[b]			X	X	X	X				X			
CBC with Differential	X[b]	X		X	X	X	X	X	X	X	X	X		

Trial Period:		**Treatment Cycles[a]**									**End of Treatment**	**Post-Treatment**		
		Cycle 1					May be repeated beyond 8 cycles							
Treatment Cycle:	Main Study Screening (Visit 1)	C1D 1	C1D 15	2	3	4	5	6	7	8		Safety Follow-up- 30 days post discon	Follow-Up Every 12 weeks up to 2yrs[i]	Survival Follow-Up[n] Annually
Days:		1	15	22	43	64	85	106	127	148	Discon[h]			
Scheduling Window (Days):	see footnotes	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	± 3	±3wks	
Comprehensive Serum Chemistry Panel[o]	X[b]	X		X	X	X	X	X	X	X	X	X		
Urinalysis		X			X		X		X					
T3, FT4 and TSH		X		X		X		X		X				
HIV/Hep C[d]	X													
Tumor Imaging - CT(or MRI) chest/abdomen/pelvis head when indicated	X[g]					X[g]				X[g]	X			
Symptom Diary		X		X	X	X	X	X	X	X	X	X		
Breast tumor biopsy[m]		X		X										
Optional tumor biopsies						X[k]								
Correlative Studies Blood Collection[l]		X		X	X	X								

a Cycles are 21 days
b Pre-study, within 10 days of registration.
c Pre-study, within 2 weeks of registration.
d HIV/Hep C testing required if known or suspected history or exposure
f Must be completed within 72 hours prior to receiving the first dose of study drug.
g Baseline tumor imaging should be within 28 days of registration. Tumor imaging is planned for within 4 days of receipt of study drug on cycle #4. Imaging is to be every 12 weeks (± 1 week) for any patients continuing on therapy beyond cycle #4.
h If a subject is discontinued and the assessments have been completed as part of another study visit, the assessments do not need to be repeated. Tumor imaging does not need to be repeated if scans have been completed within the past 8 weeks.
i Follow-up visits may be discontinued at (1) the start of new anti-cancer treatment, (2) disease progression, (3) death, (4) withdraw of consent or lost to follow-up, (5) the end of the follow-up visit period, or (6) the end of the study, whichever occurs first. Any visits for subsequent therapy may replace protocol required survival follow-up visits. Phone follow-up is acceptable.

j All AEs occurring within 30 days after the last dose of trial treatment will be recorded. SAEs (related and unrelated to trial treatment), UADEs and ECIs occurring up until 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever comes first, will be recorded. Afterwards, only SAEs, UADEs and ECIs that are related to trial treatment will be reported. The 90 day SAE collection may be collected by phone.
k Optional tumor biopsies of HIFU treated lesion and distant tumor site where clinically safe.
l Bloods for immunologic testing should be collected prior to study drug infusion and will require 4 tubes (10 ml tubes).
m Breast biopsy target is minimum of 6 core needle passes. For the cycle 2 biopsy, three of the needle passes should target the central ablated zone and three should target the peri-ablation zone.
n Survival follow-up may be completed by phone.
o Refer to Table 6 for a complete list of analyses to be completed as part of the comprehensive chemistry panel.

ARM B

Trial Period:		**Treatment Cycles[a]**									**End of Treatment**	**Post-Treatment**		
		Cycle 1					May be repeated beyond 8 cycles							
Treatment Cycle:	Main Study Screening (Visit 1)	C1D 1	C1D 15	2	3	4	5	6	7	8		Safety Follow-up-30 days post discon	Follow-Up Every 12 weeks up to 2yrs[i]	Survival Follow-Up[n] Annually
Days:		1	15	22	43	64	85	106	127	148	Discon[h]			
Scheduling Window (Days):	See footnotes	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	± 3	± 3wks	
Informed Consent	X													
Inclusion/Exclusion Criteria	X													
Demographics and Medical History	X													
Prior and Concomitant Medication Review	X	X		X	X	X	X	X	X	X	X	X		
Ultrasound	X													
Focused Ultrasound Treatment			X											
Pembrolizumab Infusion		X		X	X	X	X	X	X	X				
Post-study anticancer therapy status												X	X	X
Survival Status														X
Review Adverse Events		X	X	X	X	X	X	X	X	X	X	X[j]	X[j]	
Full Physical Examination	X[b]													
Pain Assessment (VAS Scale)			X	X										
Directed Physical Examination		X		X	X	X	X	X	X	X	X	X		
Vital Signs and Weight	X[c]	X		X	X	X	X	X	X	X	X	X		
ECOG Performance Status	X[b]	X		X	X	X	X	X	X	X	X			
Pregnancy Test – Urine or Serum β-HCG	X[f]		X											
PT/INR and aPTT	X[b]			X	X	X	X				X			
CBC with Differential	X[b]	X		X	X	X	X	X	X	X	X	X		
Comprehensive Serum Chemistry Panel[o]	X[b]	X		X	X	X	X	X	X	X	X	X		

Trial Period:		**Treatment Cycles[a]**									**End of Treatment**	**Post-Treatment**		
		Cycle 1					May be repeated beyond 8 cycles							
Treatment Cycle:	Main Study Screening (Visit 1)	C1D 1	C1D 15	2	3	4	5	6	7	8		Safety Follow-up- 30 days post discon	Follow-Up Every 12 weeks up to 2yrs[i]	Survival Follow-Up[n] Annually
Days:		1	15	22	43	64	85	106	127	148	Discon[h]			
Scheduling Window (Days):	See footnotes	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	± 3	± 3wks	
Urinalysis		X			X		X		X					
T3, FT4 and TSH		X		X		X		X		X				
HIV/Hep C[d]	X													
Tumor Imaging - CT(or MRI) chest/abdomen/pelvis head when indicated	X[g]					X[g]				X[g]	X			
Symptom Diary		X	X	X	X	X	X	X	X	X	X	X		
Breast tumor biopsy[m]		X		X										
Optional tumor biopsies						X[k]								
Correlative Studies Blood Collection[l]		X		X	X	X								

a Cycles are 21 days
b Pre-study, within 10 days of registration.
c Pre-study, within 2 weeks of registration.
d HIV/Hep C testing required if known or suspected history or exposure
f Must be completed within 72 hours prior to receiving the first dose of study drug.
g Baseline tumor imaging should be within 28 days of registration. Tumor imaging is planned for within 4 days of receipt of study drug on cycle #4. Imaging is to be every 12 weeks (± 1 week) for any patients continuing on therapy beyond cycle #4.
h If a subject is discontinued and the assessments have been completed as part of another study visit, the assessments do not need to be repeated. Tumor imaging does not need to be repeated if scans have been completed within the past 8 weeks.
i Follow-up visits may be discontinued at (1) the start of new anti-cancer treatment, (2) disease progression, (3) death, (4) withdraw of consent or lost to follow-up, (5) the end of the follow-up visit period, or (6) the end of the study, whichever occurs first. Any visits for subsequent therapy may replace protocol required survival follow-up visits. Phone follow-up is acceptable.

j All AEs occurring within 30 days after the last dose of trial treatment will be recorded. SAEs (related and unrelated to trial treatment), UADEs and ECIs occurring up until 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever comes first, will be recorded. Afterwards, only SAEs, UADEs and ECIs that are related to trial treatment will be reported. The 90 day SAE collection may be collected by phone.
k Optional tumor biopsies of HIFU treated lesion and distant tumor site where clinically safe.
l Bloods for immunologic testing should be collected prior to study drug infusion and will require 4 tubes (10 ml tubes).
m Breast biopsy target is minimum of 6 core needle passes. For the cycle 2 biopsy, three of the needle passes should target the central ablated zone and three should target the peri-ablation zone.
n Survival follow-up may be completed by phone.
o Refer to Table 6 for a complete list of analyses to be completed as part of the comprehensive chemistry panel.

Study Flow Chart- Second Course Phase

		Treatment cycles (3-Week Cycles)		End of Treatment		
		To be repeated beyond cycle 3 of second course				
Treatment Cycle/Title	**1**	**Cycle 2**	**Cycle 3**	**Discontinuation of pembrolizumab[f]**	**Safety follow-up Discontinuation of pembrolizumab**	**Follow-up visits [g]**
Scheduling Window (days)	**±3**	**±3**	**±3**	**±3**	**±3**	**±10**
				At the time of discontinuation of pembrolizumab	30 days post-discontinuation of pembrolizumab	Every 12 weeks post discontinuation up to 2 years from initiating second-course treatment or per footnote
Eligibility Criteria	X[a]					
Physical Exam/vitals[b]	X	X	X	X	X	
AE assessment	X	X	X	X	X	X[h]
Prior and concomitant medication review	X	X	X	X	X	X
Weight	X	X	X			
ECOG PS	X	X	X	X		
Pregnancy test-urine or serum β-HCG	X[c]					
CBC with diff	X	X	X	X	X	
Comprehensive chem. panel[i]	X	X	X	X	X	
T3, FT4 and TSH	X		X	X	X	
Urinalysis	X		X	X	X	
Optional Biopsy[d]	X			X		
Pembrolizumab	X	X	X			
Tumor Imaging - CT(or MRI) chest/abdomen/pelvis		per standard clinical practice or a minimum of every 12 weeks		X[e]		X[e]
Symptom Diary	X	X	X	X	X	
Post-study anticancer therapy status						X
Survival Status						

[a] Subjects who either a) attain a CR and discontinue treatment or b) discontinue treatment after 24 months on pembrolizumab for reasons other than disease progression or intolerability may restart trial treatment if they meet the criteria specified in Section 7.1.6.4.
[b] A complete physical exam should be completed at cycle 1. Symptom-directed physical exams may be conducted at all other visits.
[c] Must be completed within 72 hours prior to receiving day 1 of this second course of study drug.
[d] Optional: If subjects develop metastatic deposits accessible to biopsy/excision with minimal morbidity, a specimen may be collected.
[e]Tumor imaging will be completed every 12 weeks or per standard clinical practice. Tumor imaging does not need to be repeated if scans have been completed within the past 8 weeks.
[f] If a subject is discontinued and the assessments have been completed as part of a regularly scheduled visit, the assessments do not need to be repeated.
[g] Follow-up visits may be discontinued at (1) the start of new anti-cancer treatment, (2) disease progression, (3) death, (4) withdraw of consent or lost to follow-up, (5) the end of the follow-up visit period, or (6) the end of the study, whichever occurs first. Any visits for subsequent therapy may replace protocol required survival follow-up visits. Phone follow-up is acceptable.
[h] All AEs occurring within 30 days after the last dose of trial treatment will be recorded. SAEs (related and unrelated to trial treatment) and ECIs occurring up until 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever comes first, will be recorded. Afterwards, only SAEs and ECIs that are related to trial treatment will be reported. The 90 day SAE collection may be collected by phone.
[i] Refer to Table 6 for a complete list of analyses to be completed as part of the comprehensive chemistry panel.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Registration

All participants must sign the consent form prior to determination of eligibility for this study. All participants who meet the inclusion/exclusion criteria may be registered. Registration will occur following verification of eligibility by the treating physician. Participants should receive their first study treatment within 2 weeks of registration.

7.1.1.4 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.1.5 Prior and Concomitant Medications Review

7.1.1.5.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.5.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.6 Disease Details and Treatments

7.1.1.6.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.6.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.1.6.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.1.1.7 Assignment of Randomization Number

The randomization codes are generated by the study statisticians and stored in the UVA Cancer Center Clinical Trials Database.

7.1.1.8 Trial Compliance (Medication/Diet/Activity/Other)

Treatment compliance may be evaluated through drug accountability assessments and through the evaluation of subject medical records and CRF documents.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to Section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

7.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 11.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

Tumors will be assessed by CT scan (or MRI) of the chest, abdomen, and pelvis at baseline and at cycle 4 as per Flow Chart 6.1 above. After completion of cycle 4, imaging will be performed every 4 cycles (approximately 12 weeks) as per usual standard of care. For subjects with history of brain metastases, imaging of the head will be performed at screening to determine stability of brain metastases as indicated in Section 5.1.3 criterion 8.

7.1.2.7 Ultrasound Imaging

Ultrasound imaging will be performed during screening to confirm presence of a lesion within the Echopulse treatment parameters as indicated in Section 5.1.2 criterion 6.

7.1.2.8 Tumor Tissue Collection and Correlative Studies Blood Sampling

7.1.2.8.1 Core Biopsies

Tumor tissue biopsies will occur at baseline and at cycle 2 as per Flow Chart 6.1 above. Optional tumor biopsies are permitted at cycle 4 where deemed safe. For the core biopsies, 14-18 gauge needles may be used for tissue sampling. Ultrasound guidance or CT guidance may be used at the time of the biopsy. The tissue specimens will be collected in the following priority:

- (1) Formalin-fixed for paraffin embedding (TMA for IHC and TCR secquencing)
- (1) RPMI for single cell suspensions (flow cytometric analyses)
- (1) Trizol for RNA extraction (gene expression analyses)

Leftover samples may be banked and stored for future biomedical research

7.1.2.8.2 Blood Collection for Research Analyses

Bloods for correlative studies will be collected prior to study drug infusion at baseline and at cycles 2, 3 and 4 as per Flow Chart 6.1 above.

The following blood samples for research will be collected and processed by the UVA Biorepository and Tissue Research Facility (BTRF).

- 40 cc blood collected in heparinized green top tubes for lymphocytes.

Leftover samples may be banked and stored for future biomedical research.

7.1.2.9 Patient-Rated Pain Assessment

The pain associated with the investigational procedure will be assessed prior to the HIFU treatment and immediately following the HIFU treatment (C1D15) to evaluate pain associated with the procedure. Additional pain assessments will be made at Cycle 2. Pain will be rated by each subject using a standard Visual Analog Scale (VAS).

7.1.3 HIFU Device Performance Parameters

The following data will be collected during the HIFU treatment:

- Total duration of treatment session

- Energy settings used during procedure

7.1.4 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 6.

Table 6: Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

‡ If considered standard of care in your region.

Laboratory tests for screening or entry into the Second Course Phase should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

Sample collection, storage and shipment instructions for serum samples will be provided in the Laboratory Manual.

Sample collection, storage and shipment instructions for blood samples will be provided in the Laboratory Manual.

7.1.5 Other Procedures

7.1.5.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.6.5. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.6.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.6.4).

7.1.5.2 Blinding/Unblinding

7.1.6 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.6.1 Screening

A member of the study team will explain the purpose of the study and the study-related procedures to potential subjects. Subjects will be asked to provide written informed consent prior to the initiation of any study-related procedures. The results from assessments performed as part of a subject's clinical care prior to receipt of informed consent may be utilized to fulfill a screening requirement, if the assessments were completed with the required window for screening.

7.1.6.2 Treatment Period

The treatment period will begin on Day 1 and will continue until the subject completes the treatment regimen or discontinues/withdraws from treatment (please refer to Section 5.8).

7.1.6.2.1 End of Treatment Visit: This visit will occur at the time that a subject is discontinued from the study.

7.1.6.3 Post-Treatment Visits

7.1.6.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who receive pembrolizumab treatment beyond Cycle 4 and are eligible for retreatment with pembrolizumab (as described in Section 7.1.6.5) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.1.6.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 12 weeks (± 7 days) by radiologic imaging to monitor disease status unless the patient started on new anti-neoplastic therapy. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, or end of the study or if the subject begins retreatment with pembrolizumab as detailed in Section 7.1.6.5. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 7.1.6.5 will move from the follow-up phase to the Second Course Phase when they experience disease progression.

7.1.6.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase. Subjects who experience confirmed disease progression or who start a new anti-cancer therapy, will also move into survival follow-up and should be contacted by telephone annually to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.6.5 Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab with SD or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- **Either**
    - Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, and
        - Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy

- Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

OR

- Had SD, PR or CR and stopped pembrolizumab treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Meets the safety parameters listed in the inclusion/exclusion criteria

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

Visit requirements are outlined in Section 6.0 – Trial Flow Chart.

7.2 Assessing and Recording Adverse Events

7.2.1 Time Span for Recording Adverse Events

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of treatment allocation/randomization through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in Section 7.2.6. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

7.2.2 Definitions

Adverse Event (AE): An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure,

whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Progression of the cancer under study is not considered an adverse event.

Unexpected AE – Any adverse event not listed in Section 7.2.9.

Serious AE: A serious adverse event is any adverse event occurring at any dose during the study that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is another important medical event

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;

Unanticipated Adverse Device Effect: An unanticipated adverse device effect (UADE) is any serious adverse event on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Device Failure: A device has failed if it does not perform according to labeling and negatively impacts the treatment while used according to the labeling.

Device Malfunction: A device malfunction is an unexpected change to the device that is contradictory to the labeling and may or may not affect device performance.

Device failure and malfunctions- All investigational device failures and malfunctions will be documented on the CRF and reported in the clinical results.

User Error- User errors that result in the inability to use the device (ex., contamination) should not be reported as a device malfunctions.

Unanticipated problem - An unanticipated problem is any event/experience that meets ALL 3 criteria below:

- Is unexpected in terms of nature, severity or frequency given the research procedures that are described in the protocol-related documents AND in the characteristics of the participant population being studied.

- Is related or possibly related to participation in research. This means that there is a reasonable possibility that the incident may have been caused by the procedures involved in the research study.

- The incident suggests that the research placed the participant or others at greater risk of harm than was previously known or recognized OR results in actual harm to the participant or others.

Protocol Violation- A protocol violation is defined as any change, deviation, or departure from the study design or procedures of a research project that is NOT approved by the institution's IRB prior to its initiation or implementation, OR deviation from standard operating procedures, Good Clinical Practices (GCPs), federal, state or local regulations. Protocol violations may or may not be under the control of the study team or UVa staff. These protocol violations may be major or minor violations.

Suspected Adverse Reaction (as defined in 21 CFR 312.32 (a))- Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.

7.2.3 Attribution Assessment

Attribution – The determination of whether an adverse event is related to a medical treatment or procedure. Please refer to Table 7 for additional guidance on evaluation of attribution.

Definite – Applies to those adverse events which, the investigator feels are incontrovertibly related to study drug or HIFU procedure. An adverse event may be assigned an attribution of definitely related if or when (must have all of the following):

- It follows a reasonable temporal sequence from administration of the test drug or HIFU procedure.
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction in dose with re-exposure to drug. (Note: This is not to be constructed as requiring re-exposure of the subject; however, the group of definitely related can only be used when a recurrence is observed.)
- It follows a known pattern of response to the test drug or HIFU procedure.

Probable – Applies to those adverse events for which, after careful consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test drug or HIFU procedure. An adverse event may be considered probably related if or when (must have three of the following):

- It follows a reasonable temporal sequence from administration of the test drug or HUIFU procedure.
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists (e.g. bone marrow depression, fixed drug eruptions, tardive dyskinesia).
- It follows a known pattern of response to the test drug or HIFU procedure.

Possible – Applies to those adverse events for which, after careful consideration at the time they are evaluated, a connection with the test drug administration or HIFU procedure appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when (must have two of the following):

- It follows a reasonable temporal sequence from administration of the test drug or HIFU procedure.
- It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It follows a known pattern of response to the test drug or HIFU procedure.

Unlikely – Applies to those adverse events for which, after careful consideration at the time they are evaluated, are judged to be unrelated to the test drug or HIFU procedure. An adverse event may be considered unlikely if or when (must have two of the following):

- It does not follow a reasonable temporal sequence from administration of the test drug or HIFU procedure.
- It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the test drug or HIFU procedure.
- It does not reappear or worsen when the drug is re-administered.

Unrelated – Applies to those adverse events, which after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).

7.2.4 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose

of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.5 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.6 Immediate Reporting of Adverse Events to the Sponsor and to Merck

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study whether or not related

to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

All subjects with serious adverse events must be followed up for outcome.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

7.2.6.1 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.3 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

> *Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.2.6.2 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section7.2.6.- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g.transportation issues etc.) will not be considered a SAE.

7.2.7 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 7: Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days..	

	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck Product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponsor's product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).**	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.	
No, there is not a reasonable possibility of Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (Also entered for a subject with overdose without an associated AE.)	

7.2.8 Adverse Event Classifications

Adverse events (AEs) are classified into sections, specified in the CTCAE v4.03. For specific classifications pertaining to the protocol, we specify the following:

Hematologic/Metabolic- Any AE coded under one of the following CTCAE v4.03 categories should be reported under the Hematologic/Metabolic adverse event classification:

Table 8: Hematologic/Metabolic Classifications

Section	AE
Blood and lymphatic	Anemia Leukocytosis
Investigations	**ALL EXCEPT:** Carbon monoxide diffusing capacity decreased Ejection fraction decreased Forced expiratory volume decreased Vital capacity abnormal Weight gain Weight loss
Metabolism and nutrition disorders	**ALL EXCEPT:** Alcohol intolerance Anorexia Dehydration Glucose intolerance Iron overload Obesity Tumor lysis syndrome

Non-hematologic/Non-Metabolic- Any AE not reported under hematologic/metabolic, ocular, or allergic/autoimmune, should be reported under the non-hematologic/non-metabolic adverse event classification.

Allergic/Autoimmune – Only AEs coded as Immune System Disorder: Allergic reaction, autoimmune disorder, or anaphylaxis should be reported under the Allergic/Autoimmune adverse event classification. Other AEs coded under Immune System Disorder should be reported under Non-hematologic/Non-metabolic.

7.2.9 Agent-Specific Expected Adverse Events List

7.2.9.1 Pembrolizumab

Treatment-related adverse reactions are described in Section 7 of the Investigator's Brochure.

7.2.9.2 Adverse Effects Considered Expected for HIFU

Data for potential adverse effects related to the HIFU treatment are described in the Echopulse User's Manual.

7.2.9.3 Adverse events expected from biopsies

Adverse events expected from biopsies (highest grade expected—Grade 2)

- Bleeding
- Bruising
- Pain
- Lymphedema
- Delayed wound healing
- Scarring
- Numbness
- Gross hematuria and/or dysuria and urinary frequency (prostate biopsies)
- hematochezia (prostate biopsies)
- Infection

7.2.10 Dose Limiting Toxicities

A DLT of pembrolizumab is defined as an unacceptable, drug-related toxicity requiring permanent discontinuation, per Table 4 (Dose Modification Guidelines for Drug-Related Adverse Events).

7.2.11 Recording and Reporting Adverse Events

7.2.11.1 Process for Recording Adverse Events

Dose-limiting toxicities (DLTs)

DLTs will be entered into Oncore within 5 calendar days of the study team learning of the event. DLTs that are deemed serious and unexpected will be submitted to the IRB per institutional guidelines (see below).

Other AEs

AEs must be recorded into the University of Virginia Cancer Center OnCore database per the following guidelines:

Table 9: Recording AEs into the OnCore Database

High Risk Studies Reporting requirements for AEs that occur within 30 days of the last dose of protocol specified treatment								
	Grade 1	Grade 2		Grade 3				Grade 4 & 5
	Expected and unexpected	Expected	Unexpected	Expected		Unexpected		Expected and Unexpected
				Without hospitalization	With hospitalization	Without hospitalization	With hospitalization	
Unrelated Unlikely	OnCore 30 days[a]	OnCore 30 days	OnCore 30 days	OnCore 30 days	OnCore 15 days	OnCore 30 days	OnCore 15 days	OnCore 7 days
Possible Probable Definite	OnCore 30 days[a]	OnCore 30 days	OnCore 15 days	OnCore 30 days	OnCore 15 days	OnCore 7 days	OnCore 7 days	OnCore (24-hrs)* 7 days

*Enter into OnCore database within 24 hours if unexpected and definitely related to protocol specified treatment
Hospitalization defined as an inpatient hospital stay or prolongation of a hospital stay equal to or greater than 24 hours
[a] Grade 1 unexpected or expected hematologic/metabolic events will be recoreded in the Cancer Center Database; however, regardless of attribution, these events do not have to be reported.

7.2.11.2 Recording Laboratory Values

Laboratory values specified in Table 6 will be recorded in the UVA Cancer Center database, graded using the CTCAE v4.03 (if a grading category exists) and reported as described in Section 7.2.11. Any abnormal laboratory values captured which are not included in the list, but are considered to be pertinent positive clinical signs/symptoms will be recorded in the UVA Cancer Center database and reported as described in Section 7.2.11. If there is any doubt on the part of study personnel concerning what constitutes a pertinent positive finding, the Sponsor-Investigator will be consulted.

7.2.11.3 UVA IRB Reporting Requirements

The University of Virginia is responsible for reporting to the UVA IRB-HSR per the following guidelines:

Table 10: UVA IRB-HSR Reporting

Type of Event	To whom will it be reported:	Time Frame for Reporting	How reported?
Any internal event resulting in death that is deemed DEFINITELY related to (caused by) study participation (Note: An internal event is one that occurs in a subject enrolled in a UVa protocol.)	IRB-HSR	Within 24 hours	IRB Online and phone call www.irb.virginia.edu/
Internal, Serious, Unexpected adverse event.	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event. *Timeline includes submission of signed hardcopy of AE form.*	IRB Online www.irb.virginia.edu/
For Device Studies: Unanticipated adverse device effects (internal)	IRB-HSR	Within 10 day calendar days of the study team receiving knowledge of the event	IRB Online www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations This would include a Data Breach.	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. *http://www.virginia.edu/vprgs/irb/HSR_docs/Forms/Reporting_Requirements-Unanticipated_Problems.doc*
Protocol Violations (*The IRB-HSR only requires that MAJOR violation be reported, unless otherwise required by your sponsor, if applicable.)* Or Enrollment Exceptions	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation and Enrollment Exception Reporting Form *http://www.virginia.edu/vprgs/irb/hsr_forms.html*
Data Breach	The UVa Corporate Compliance and Privacy Office and ITC: if breach involves electronic data- UVa Police if breach includes such things as stolen computers.	As soon as possible and no later than 24 hours from the time the incident is identified. As soon as possible and no later than 24 hours from the time the incident is identified. IMMEDIATELY.	UVa Corporate Compliance and Privacy Office- Phone 924-9741 ITC: Information Security Incident Reporting procedure, http://www.itc.virginia.edu/security/reporting.html Phone- (434) 924-7166

7.2.11.4 Reporting to the FDA

The Sponsor-Investigator for the study (the UVA PI or designee) is responsible for providing safety updates to the FDA per the following guidelines. The reporting times refer to the time the study team received knowledge of the event.

FDA Reporting Requirements for Pembrolizumab related adverse events

- Serious and unexpected suspected adverse reactions will be reported to the FDA no later than 15 calendar days after the sponsor determines that the requirements for an IND safety report have been met. The FDA will be notified using an FDA Form 3500a.
- Unexpected fatal or life-threatening suspected adverse reactions will be reported to the FDA no later than 7 calendar days after the Sponsor receives the initial information of the event. The FDA will be notified using an FDA Form 3500a.

Other adverse event information will be sent to the FDA in the IND annual report.

FDA Reporting Requirements for HIFU: Unanticipated adverse device effects (UADE)

- Unanticipated adverse device effects (UADEs) will be reported to the FDA no later than 10 calendar days after the Sponsor-Investigator receives the initial information of the event.

Other adverse event information will be sent to the FDA in the IND annual report.

7.2.11.5 Reporting to the Echopulse Manufacturer

All unanticipated adverse device effects (UADEs) will be reported to the Echopulse Manufacturer (Theraclion) no later than 5 calendar days after the Sponsor-Investigator receives the initial information of the event.

7.2.12 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

This is a pilot study of high intensity focused ultrasound (HIFU) and pembrolizumab, and is proposed as a pilot two-arm design. All patients will receive the FDA approved dosing of pembrolizumab and allocation to arms is randomized. Participants from arm A will first receive HIFU ablation and then a pembrolizumab infusion 1 week after an ablative procedure. Participants from arm B will first receive pembrolizumab at baseline and then undergo the HIFU ablation two weeks later. The goal is to obtain preliminary data on the safety of HIFU and pembrolizumab and to obtain estimates of change in immune parameters (proportion of activated CD8 tumor infiltrating lymphocytes (TILs) is primary) pre- and post-treatment. The trial will not be powered for cross arm comparison, but will nevertheless provide useful pilot data for the design of a larger trial of combined therapies.

Safety stopping rules will be employed within each study arm and will be judged by dose limiting toxicities (DLTs), defined in Section 7.2.10. The primary objectives include assessing adverse events

and change in the proportion of $CD8^+$ to $CD4^+$ tumor infiltrating lymphocytes (TILs) in the primary ablation zone. Secondary endpoints include assessing change in $CD8^+$ T-cell responses at peri-ablation zones, clinical responses at local and distant metastatic sites, disease free survival (DFS), and overall survival (OS). Exploratory endpoints include assessing change in T-cells in peripheral blood, markers of T-cell activation, gene expression profiling, and enumeration of myeloid derived suppressor cells.

8.2 Statistical Analysis Plan

Target sample size is 12 participants, which was chosen as a feasible number that could be accrued within a 2 year accrual period. Participants will be randomized 1:1 to treatment arms A and B with block randomization using blocks of size 2 or 4. Participants are considered evaluable if they satisfy all inclusion and exclusion criteria and have adequate endpoint measurements during respective cohort timelines. Adjusting for a 10% unevaluable/drop-out rate, maximum target accrual is estimated at 15 participants.

The purpose is to assess the adverse events profile of pembrolizumab and focused ultrasound therapy and estimate the difference in the TILs at baseline and 3 weeks within each arm, not to make definitive comparisons between arms. With 6 participants per arm, there is limited power to make comparisons between arms. Lu et. al. (2009) data estimates the difference in means of the ratio of $CD4^+$ and $CD8^+$ cells for patients treated with HIFU versus no HIFU to be 0.57, with standard deviations ranging from 0.27 to 0.40. These data provide an indication of the magnitude that could be detected in this study at baseline and 3 weeks, where it is assumed that the no HIFU participants in Lu et. al. are comparable to baseline measurements in our study. Using these data as a guideline, with n=6, we have 80% power to detect an effect size of 1.44 for the within-arm change in ratio of $CD4^+$ and $CD8^+$ cells at baseline and 3 weeks. Depending on the variability of the data, this is equivalent to detecting differences of 0.39 and 0.58, for assumed standard deviations of 0.27 and 0.40, respectively. The populations will include all intention to treat participants.

Safety stopping rules will be employed within each arm and will be judged by dose limiting toxicities (DLT). If the first two participants within an arm or 3 out of the 6 participants within an arm experience a DLT, that arm will be deemed too toxic and further accrual to that arm will be closed. This decision rule is based on Wald's sequential probability ratio test assuming lower and upper DLT proportions of 0.1 and 0.3, respectively, and type I and II error rates of 10%.

Adverse events and DLTs will be summarized by frequency and magnitude for each arm and overall. Change in the proportion of $CD8^+$ to $CD4^+$ tumor infiltrating lymphocytes (TILs) in the primary ablation zone will be estimated by arm. Additionally change in $CD8^+$ T-cell responses at peri-ablation zones and clinical response at local and distant metastatic sites measured by abscopal tumor shrinkage at non-ablated sites, will be estimated for each arm. The Kaplan Meier estimator will be used to estimate survival curves for DFS and OS. Exploratory endpoints including change in T-cells in peripheral blood, markers of T-cell activation, gene expression profiling, and enumeration of myeloid derived suppressor cells will be estimated by arm.

If highly favorable responses are seen in this pilot patient population, then this study may be expanded by means of an amendment to more rigorously define response criteria in either or both arms.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product and Device

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product and device in accordance with the protocol and any applicable laws and regulations.

Clinical drug supplies will be provided by Merck as summarized in Table 8.

Table 8: Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 50 mg	Lyophilized Powder for Injection
Pembrolizumab 100 mg/ 4mL	Solution for Injection

Theraclion will provide the Echopulse device and EPacks, a cooling and coupling set that is used to enable wave transmission and to protect the skin during the HIFU treatment. EPacks are composed of a special liquid, tubing and a membrane and are described further in the user manual.

9.2 Packaging and Labeling Information

Clinical supplies (drug and device) will be affixed with a clinical label in accordance with regulatory requirements.

Echopulse and ePack labeling information can be found in the Echopulse user manual.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

9.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical drug supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Study Conduct and Ethical Considerations

This study will be conducted in accordance with the standards of Good Clinical Practice, all applicable federal, state, and local laws, and in accord with the ethical principles that originated in the Declaration of Helsinki. The Principal Investigator (PI) is responsible for the conduct of the clinical trial at the site and the PI will ensure that staff are trained and carry out the study in accord with the protocol specifications. The PI will ensure that all study site personnel are aware that the study protocol and all data generated are confidential and should not be disclosed to third parties (with the exception of local and national regulatory bodies which require access for oversight purposes).

10.2 UVA Institutional Review Board for Health Sciences Research

The UVA Institutional Review Board for Health Sciences Research (UVA IRB-HSR) will approve all aspects of this study, including the clinical trial protocol, informed consent documents, and patient materials. Modifications to the protocol or consent form will be reviewed and approved by the UVA IRB-HSR prior to implementation, except when necessary to eliminate apparent immediate hazards to the study participants. The study will undergo continuing IRB review based on the level of risk as assessed by the IRB. This review will take place no less than annually. Reporting to the UVA IRB-HSR will occur as specified in Section 7.2.7.

10.3 Consent Forms and the Consenting Process

Consent forms will be written in accord with 21 CFR 50 and will be reviewed and approved by the UVA IRB-HSR prior to use. Participants will be given a consent form to review and a member of the study team will be available to answer any questions. Informed consent will be obtained from each participant prior to conducting any study-specific procedures or administering study drug.

10.4 Maintenance of Study Documents

Study documents and records will be retained in accordance with local IRB requirements and the regulations specified in 21 CFR 312.

10.5 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.6 Data Collection

Data will be collected using a centralized electronic case report form called **ON**-line **C**linical **O**ncology **R**esearch **E**nvironment = **Oncore**.

10.6.1 Endpoint Data

Endpoint data will be collected using HITC IML data forms, participant-specific binders, and the HITC laboratory database.

The HITC laboratory database, which has password-restricted access, is stored on the UVA Health System Computing Services secured server.

10.7 Data Safety Monitoring Plan

The University of Virginia Cancer Center Data and Safety Monitoring Committee (CC DSMC) will provide oversight of the conduct of this study. The CC DSMC will report to the UVA Protocol Review Committee (PRC).

The UVA CC DSMC will review the following:

- All adverse events
- Audit results
- Application of study designed stopping/decision rules
- Whether the study accrual pattern warrants continuation/action
- Protocol violations

10.8 DSMC Monitoring Schedule

The UVA CC DSMC will meet every month for aggregate review of data. Tracking reports of the meetings are available to the PI for review. Issues of immediate concern by the DSMC are brought to the attention of the sponsor (and if appropriate to the PRC and IRB) and a formal response from the sponsor is requested. Per the UVA Cancer Center NIH approved institutional plan, this study will be audited approximately every 6 months.

11.0 APPENDICES

11.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

11.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

11.3 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

11.4 Summary of Changes

Version Date	Description of Changes
04-13-21	Section 4.3: Updated the rationale for pembrolizumab dose selection per updated Merck protocol template language Section 5.1.3: Added to exclusion criterion #10 to state that subjects must not have a history of interstitial lung disease or have current interstitial lung disease. Added to exclusion criterion #18 to state that subjects must not have received a live attenuated vaccine within 30 days of planned start of study therapy Section 5.2.2: Revised dose modification table and toxicity management table (Table 4) per updated Merck protocol template language Section 5.6.1: Revised supportive care guidelines per updated Table 4
03-12-21	Section 5.1: Clarified exclusion criterion #19 to state that HIFU must not be applied within 10mm of an implant. Section 5.2.1.2: Clarified that approximately 50% of the tumor volume should be treated with focused ultrasound ablation Section 6.1: Removed footnote "e" from the study flow chart to reflect available footnotes.
08-28-19	Section 4.2.2: added rationale to address inclusion of patients who experience disease progression by rising tumor marker and patients who are intolerant of standard therapy. Section 5.1.2: added text to inclusion criteria 4 to allow patients who experience disease progression by rising tumor marker and patients who are intolerant of standard therapy.
05-07-19	Section 5.1.2: defined chest wall per NCI.
11-30-17	Section 5.2.2: replaced Table 4 with revised Merck provided table "Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab", edited to include language regarding AEs resolving to "grade 0-1 or baseline" under general instructions #2.
11-20-17	1. Replaced references to week time points with day or cycle for consistency throughout protocol. 2. Section 2.2: replaced trial diagram with updated diagram including day and cycle timepoints and reference to HIFU treated lesion and distant tumor site for optional biopsies during cycle 4. 3. Section 5.1.2: edited criterion 9 to allow for ECOG performance status of 2 4. Section 5.1.3: corrected criterion 8 to indicate brain imaging for subjects with history of brain metastases are to be "within" four weeks of "registration" 5. Section 5.2.2: edited table 4 to include the phrase "or baseline" under the "All Other Drug-Related Toxicity". This change will allow patients to restart treatment if they resolve to baseline. 6. Section 6.1: -added ultrasound imaging at screening -added "head when indicated" regarding brain imaging for subjects with history of brain metastases.

	-removed footnote "e Focused Ultrasound treatment should take place on day 15 of cycle 1 (±2 days)" as it conflicts with intended HIFU treatment window ± 3 days indicated in flow chart header. -clarified optional biopsies "of HIFU treated lesion and distant tumor site" in footnote "k" 7. Section 7.1.2.6: added statement regarding head imaging at screening for subjects with history of brain metastases. 8. Added section 7.1.2.7 to address screening ultrasound to confirm lesion to receive HIFU treatment within the Echopulse treatment parameters.
9-28-17	Section 5.1.2: removed text "all screening labs should be performed within 10 days of treatment initiation" from exclusion criteria 10 as it conflicts with section 6.1 footnote "b" indicating that screening labs should be completed within 10 days of registration which is the intended timeline.
8-24-17	Section 6.1: -Arm A & B study chart: footnote "h" was moved to the discontinuation visit header and reworded to indicate "If a subject is discontinued and the assessments have been completed as part of another study visit, the assessments do not need to be repeated. Tumor imaging does not need to be repeated if scans have been completed within the past 8 weeks." - Second course phase study chart: footnote "e" was updated to indicate that tumor imaging would not need to be repeated if done within 8 weeks and footnote "f" redundant text regarding tumor imaging was removed.
08-14-17	1. Updated Table of Contents 2. Section 3.3: removed reference to lab manual. 3. Section 4.4.1: removed references to lab manual and referenced section 7.1.2.7 4. Section 5.2.1.2: removed proprietary information figure 4, figure 5, and corresponding text. 5. Section 6.1: -Arm A study chart: added Day 15 AE assessment -Arm A & B study charts: added day 15 pregnancy test, 4 week imaging window in footnote "h", text indicating that the 90 day SAE collection may be completed by phone in footnote "j", and footnote "o" referring to Table 6 for the complete list of comprehensive chemistry labs. -Second course phase study chart: added cycle 1 thyroid test, text indicating that the 90 day SAE collection may be completed by phone in footnote "h", and footnote "i" referring to list of comprehensive chemistry labs. -Added study day number and imaging sites to all study calendars. 6. Section 7.1.2.6: added language to specify imaging sites. 7. Added sections 7.1.2.7.1 and 7.1.2.7.2 to describe tissue and blood analyses.
06-19-17	1. Corrected hyperlinks throughout the study document. 2. Updated Table of Contents 3. Section 7.2.6-removed reference to section 7.2.3.3 and reference to section 7.2.5.2.
06-13-17	1. Updated Table of Contents 2. Section 5.1.1: revised entry criteria to clarify that the intent is to enroll patients with metastatic or unresectable breast cancer. 3. Section 5.1.2 (#3): revised entry criteria per FDA recommendation to specify that patients who are HR^+ should also no longer be candidates for hormonal-based

	therapy. Patients who are $HER2^+$ should have progressed on or no longer be candidates for available HER2 directed therapy. 4. Section 5.1.2(#5 and #6): added "chest wall" to the disease description. 5. Section 5.2.1.2: • Revised to clarify that HIFU will be administered to sites of disease located in the breast/chest wall/axilla. • Revised to add the following statements per FDA recommendation a) To mitigate damage to the rib cage, the clinician will confirm that the rib cage is not in the prefocal ultrasound path or behind the focal point (minimum distance behind the focal point = 10 mm). b) As the risk of HIFU on the lung parenchyma is not known at this time, for those patients treated with chest wall lesions the beam energy should always be oriented so that it does not reach the lung. c) Following the HIFU procedure, local application of ice may be applied to the affected area. 6. Sections 6.1: • Added Pain Assessment (VAS Scale) to the study calendars (C1D15 and C2) per FDA recommendation. • Footnote "m": revised to clarify that for the biopsies three of the needle passes should target the central ablated zone and three should target the peri-ablation zone. 7. Section 7.1.1.6: Deleted the following text to be consistent with the revisions to the entry criteria: Participants who reach week 10 of pembrolizumab treatment and will be continuing with pembrolizumab treatment may receive hormonal or HER2 therapy concomitantly if indicated. 8. Section 7.1.2.7: Revised to provide more details about the biopsies (e.g. gauge of needles and use of ultrasound or CT guidance). Tumor tissue biopsies will occur at baseline and at cycle 2 as per Flow Chart 6.1 above. Optional tumor biopsies are permitted at cycle 4 where deemed safe. For the core biopsies, 14-18 gauge needles may be used for tissue sampling. Ultrasound guidance or CT guidance may be used at the time of the biopsy. 9. Section 7.1.2.8: Added the following text to describe the patient-rated pain assessments: The pain associated with the investigational procedure will be assessed prior to the HIFU treatment and immediately following the HIFU treatment (C1D15) to evaluate pain associated with the procedure. Additional pain assessments will be made at Cycle 2. Pain will be rated by each subject using a standard Visual Analog Scale (VAS).

	10. Editorial corrections (e.g. spacing, font, spelling) corrected throughout the study protocol.
05-15-17	1. Updated Table of Contents 2. Updated Schema 3. Corrected typographical errors in Sections 5.2.1.2, 5.2.3, and 5.8. 4. Section 6.1: removed footnotes "b" informed consent, inclusion/exclusion, demographics, history and medication review at screening as the 10 day window does not apply. Clarified that baseline imaging should be completed within 28 days of registration in footnote "g". 5. Section 7.2.1: removed duplicate language. 6. Section 7.2.3: edited to include HIFU procedure in attribution assessments. 7. Section 7.2.6: removed repetitive language addressed in section 7.2.2. 8. Section 9: edited to include Echopulse device.
05-03-17	1. Added IND number to the Title Page 2. Updated Table of Contents 3. Corrected typographical errors in Sections 6.1, 7.2.9.2, and 7.2.11.5 4. Edited formatting for consistency throughout protocol.
05-01-17	1. Updated Table of Contents 2. Sections 5.2.1.1 and 5.2.3: removed pharmacy manual references and refer to standard practice for pembrolizumab preparation and administration. 3. Section 5.2.1.2: added detail regarding the HIFU treatment procedure. 4. Section 6.1: removed "of pembro" from end of treatment and safety follow up, divided follow-up and survival follow-up into separate columns in the study flow chart, added UADEs to footnote "j" and added footnote "n" indicating that survival follow up may be completed by phone. 5. Section 7.1.3: added section to address HIFU device performance parameters 6. Section 7.2.2: added definitions for unanticipated adverse device effects, device failure and malfunctions. 7. Section 7.2.9.2: referred to the Echopulse user manual for expected adverse effects. 8. Sections 7.2.11.3, 7.2.11.4 & 7.2.11.5: added UADE reporting requirements. 9. Clarified reference to HIFU treatment area in cubic centimeters throughout
03-27-17	1. Section 7.1.5.5: edited to match Merck IIT language regarding second course treatment eligibility following complete response
02-17-17	1. Updated Table of Contents page numbers. 2. Section 2.1: defined HIFU. 3. Section 2.2: clarified that subjects may have an optional biopsy at week 10. 4. Section 3: primary objective regarding adverse event profile shifted to secondary objectives. Secondary objectives shifted to exploratory objectives. 5. Section 4.1.1: updated to reflect pembrolizumab current approved indications. 6. Section 4.4: shifted to reflect adverse event profile moving to secondary endpoint. 7. Section 5.1.2: clarified that subjects must have at least one list of therapy in the metastatic setting. 8. Sectrio.5.1.3: clarified permitted steroid use, pneumonitis and implant exclusion. 9. Section 5.2.2: Table 4 edited to match current Merck template, discontinuation for grade 4 diarrhea/colitis.

	10. Sections 5.8.2 and 7.1.5.3.1: edited to indicate subjects must receive pembrolizumab for a minimum of 10 weeks to be considered for retreatment. 11. Section 6.1: edited the study flow chart to indicate imaging is to be completed at cycle 8 (not 7) and that correlative bloods should be collected prior to study drug infusion. 12. Section 7.1.1.5.2: added a statement that subjects continuing to receive pembrolizumab beyond week 10 may receive hormonal or HER2 therapy. 13. Section 7.1.2.6: edited to indicate imaging will be performed every 4 cycles. 14. Section 7.1.5.5: edited to match current Merck template regarding requirements for retreatment. 15. Section 8.2: edited to indicate that analysis will include discontinued subjects. 16. Corrected minor typographical errors throughout the document.
12-20-16	1. Updated Table of Contents 2. Section 5.8.3: added a new section to address pembrolizumab treatment in subjects experiencing stable disease or partial response. 3. Section 7.1.2.7: added a statement addressing correlative blood samples. 4. Section 7.1.5.5: removed separate second course treatment inclusion language and refer to inclusion/exclusion criteria. 5. Section 7.2.3: added a new section to define adverse event levels of attribution (unrelated, unlikely, possibly, probably, definite). 6. Editorial changes and correction were made throughout the study document.
11-09-16	1. Added signature page 2. Updated Table of Contents 3. Sections 2.1, 2.2, 6.1: Revised language for the biopsy collection to clarify that 1) optional biopsies of distant tumor sites may occur at week 10 and 2) additional biopsies of the HIFU treated lesion may also be taken at week 10. 4. Section 2.2, Table 3, Section 6.1, Section 8.1 Moved HIFU administration from week 2 (day 8) to week 3 (day 15). 5. Sections 4.4.1, 8.2: Deleted requirement for having the PD-L1 testing completed by Qualtek. PD-L1 testing will not be a required test for this study. 6. Section 6.1: Study Flow Chart • Removed scheduling window from the screening visit and reference the footnotes section. • Added prior and concomitant medication review to the safety follow-up visit • Revised the day for HIFU—C1, D15 • Added a pregnancy test at cycle 2 (Arm A) • Added a line for the symptom diary • Clarified language for the optional tumor biopsies • Revised footnote i (Arms A and B) and footnote g (second course treatment) so that the 2 footnotes are consistent. • Clarified in footnote l that bloods for immunologic testing will be collected in 10 ml tubes. 7. Section 8.2: Revised accrual period to 2 years. 8. Editorial changes and corrections were made throughout the study document.

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